NPYY1 /GALANIN 2 HETERODIMERS IN THE AMYGDALA: A PROXIMITY LIGATION ASSAY STUDY

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We have shown that Galanin(GAL) interacts with Neuropeptide Y Y1 receptor(Y1) at behavioural, cellular and receptor levels in the Amygdala. Since both peptides play an important role in mood disorders, the aim of this work was to study the GALR2/Y1 heterodimer formation within the amygdala(AMY), key nucleus for fear, mood, and motivation. We performed in situ proximity ligation assay(PLA) in the Amygdala and also in HEK-293 cells. Amygdala sections were incubated with anti-GALR2 Rabbit(1:100) and anti-NPYY1R Goat(1:200). In transiently transfected HEK293T cells coexpressing Y1R(3xHA) and GALR2 mouse anti-HA(1:5000) and rabbit anti-GALR2(1:500) were used. In both cases PLA signals were detected with PLA PLUS or MINUS probes for rabbit or goat/mouse antibodies. PLA signals were visualized by using a confocal microscope Leica TCS-SL confocal microscope(Leica). PLA-positive red clusters were found in large number of cells of the Basolateral, Intercalated and Central nuclei of the Amygdala. No PLA clusters were observed in lateral corpus callosum, an area that seems to

lack of GALR2 receptor. In HEK293T cells we reproduced the results obtained in the Amygdala, showing PLA-positive red clusters in large number of cells. The fact that we found PLA-positive red clusters in a large number of cells not only in the Amygdala but also in the HEK293T cells line give indications that GALR2 and NPYY1R are forming GALR2/NPYY1R heterodimers, providing a novel mechanism with implications in behavior, particularly in the anxiety response. This study was supported by Spanish CVI6476.